

[COMMUNICATION]

The Effect of Cage-Mates on Aggression in Male Mice

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ABSTRACT—Male ICR-strain laboratory mice (*Mus domesticus*) which had been reared with a castrated male from 35 to 105 days of age were paired with male mice which had been reared with an intact male from 35 to 105 days of age. Males reared with a castrated male dominated males reared with an intact male, and the former behaved more aggressively towards castrated males than the latter.

Males reared with a castrated male until 70 days of age and then reared with an intact male were paired with males which had been reared in the reverse order. The former dominated the latter. The result suggests that cage-mates under 70 days of age are important to determine social dominance.

Aggressiveness of males reared with a castrated male in cages which were divided into two compartments by a wire gauze wall was investigated. Males which grew up in a cage where the other compartment was kept empty dominated males which grew up with another pair over the wall. The result suggests that intact males affect cage-mates without physical contact.

INTRODUCTION

It is well known that rearing conditions affect the aggressiveness of male mice [1, 2]. When male mice are isolated from other males, they become more aggressive than collectively housed mice [1-4]. Individual housing, however, induces not only aggression but also other behavioral and physiological changes. Isolated mice show hyper-reaction to stimuli [5, 6]. This does not appear in collectively housed mice (irrespective of their social status). A difference is also found in the levels of some hormones [7, 8]. It is still unknown whether the isolation causes the aggressive behavior directly or increases aggressiveness as the result of physiological changes. Namikas *et al.* [9] found that males reared as the only male among five litter females became more aggressive than those reared with five males. This suggests that aggressiveness can also be induced without isolation.

There are various reports concerning the effects of cage-mates. Lagerspetz and Sandnabba [10]

report that aggressiveness is reduced by encounters between cage-mates. Some investigators, however, consider that nontactile stimuli such as olfactory substances cause the reduction in aggression [3, 11].

In the present study, intact and castrated males were used to investigate the effects of cage-mates, the period of sensitivity to the cage-mate and the properties of the stimulus involved.

MATERIALS AND METHODS

Mice of the ICR-JCL strain were housed at $22 \pm 1^\circ\text{C}$ (humidity $50 \pm 3\%$) under a 14L-10D light cycle. At 35 days of age, each of 22 intact male mice, designated "C-mates", was housed with a castrated male of the same age (35 days old) in a plastic cage measuring $16 \times 23 \times 12$ cm. Another 22 intact males, designated "I-mates", were allocated into 11 pairs and used as subjects which had been reared with an intact male. The animals were put into a clean cage every week until they reached 105 days, and then they were housed individually and exposed to strange male odor for two weeks. During the period all the males were moved every

day except the 7th day to different cages which had previously contained a strange intact male. On the 7th day of isolation, all the cages were exchanged for clean ones. After the 2-week isolation, 10 out of 22 C-mate males were tested with 10 I-mate males. Behavioral tests were conducted in a box (20×30×12 cm) which was made of plastic with a wire lid and divided into two compartments by a plastic wall. Each C-mate male was put into one compartment and an I-mate in the other for 10 min and then the wall was removed for 20 min during which aggressive behavior was observed to discern dominant-subordinate relationship.

The other 12 C-mate and 12 I-mate males were each tested with a castrated male. After the 2-week isolation, each male was put into a clean plastic cage measuring 22×15×12 cm with a wire lid for 30 min, and then a strange 119 day old castrated male which had been castrated at 35 days was introduced into the cage for 20 min. Aggressive behavior during the period was recorded.

The effect of the cage-mate was further investigated through two additional tests. In the first of these, 10 males reared with a castrated male from 35 to 70 days of age and with an intact male from 70 to 105 days of age were compared with 10 males reared with an intact male from 35 to 70 days of age and with a castrated male from 70 to 105 days of age. The former was designated as C-I and the latter as I-C. After the 2-week isolation, comparisons were made between I-C and C-I males, as the test between I-mates and C-mates.

The second test, which was intended to investigate the nature of the stimulus from the cage-mate, involved males which had grown up in cages (16×23×12 cm) partitioned longitudinally into two similar compartments by a wire gauze wall. Each male of one group (N=9), designated "No-neighbor", was housed with a castrated male in one of the compartments, and the other compartment was kept empty. Each male of the other group (N=9), designated "Neighbor", was also reared with a castrated male but the neighboring compartment housed another intact male and castrated male. Thus the Neighbor group comprised two pairs of intact and castrated male in a single partitioned cage until 105 days of age. After the 2-week isolation, No-neighbor males were tested

with Neighbor males as the test between I-mates and C-mates.

The behavioral categories noted were Attack and Fight. "Attack" refers to one-sided aggressive behavior from a specified individual, including rough grooming, chasing and biting without any counterattack from the other male. "Fight" indicates reciprocal aggressive action with neither mouse appearing to dominate the other. Fight usually includes mutual biting or wrestling. A fighting or attacking bout was considered to end when the animals parted from each other.

The male which made the more Attacks of the two was defined as the winner. There were very rare cases where both males initiated Attacks. Statistical significance was evaluated by the χ^2 test or Mann-Whitney U test. Videotape recorders were used to confirm the observations.

RESULTS AND DISCUSSION

The win-loss records are shown in Table 1. Nine C-mate males dominated I-mate males and one pair showed only Fight ($\chi^2=9.0$, $P<0.01$). In test between intact males and castrated males, castrated males did not show any aggressive activity. Therefore, numbers of Attacks shown by C-mate males and I-mate males were compared. C-mate males showed more frequent Attacks towards a castrated male than I-mate males did. The mean number (\pm SE) of Attacks was 15.7 ± 3.5 by the former and 4.7 ± 1.8 by the latter (Mann-Whitney U test, $U=12$, $P<0.01$).

The results of two other comparison are also shown in Table 1. Nine C-I males were apt to dominate I-C males and one pair ended in a tie ($\chi^2=9.0$, $P<0.01$). All the Neighbor males were dominated by No-neighbor males ($\chi^2=7.0$, $P<0.01$).

TABLE 1. Dominant-subordinate relationships between groups

Subject	Oopnent	N	No. of Subject			
			won	tie	lost	
C-mate	I-mate	10	9	1	0	$P<0.01$
C-I	I-C	10	9	1	0	$P<0.01$
No-neighbor	Neighbor	7	7	0	0	$P<0.01$

The fact that C-mate males dominated I-mate males and behaved more aggressively towards castrated males seems to imply that intact males reduce the aggressiveness of cage-mates. Lagerspetz and Sandnabba [10] proposed that aggressive behavior towards cage-mates provokes counterattacks which punish the aggressive behavior and that the males thereby learn to restrain their attacks on other males. In the present experiment, however, all the I-mate males were concurrently cage-mates of an other I-mate male. Therefore, about half of the males were probably dominant before testing [12]. Considering that the subordinates did not counterattack, it can hardly be thought that these dominant males restrained their aggressive behavior because of the counterattack of a subordinate cage-mate.

The comparison between No-neighbor and Neighbor males seems to indicate one of the factor(s) which made C-mate males dominate I-mate males. The Neighbor males which became subordinate to No-neighbor males had not physical contact with adjacent intact males. Therefore, something other than tactile stimuli from adjacent intact males must have diminished their aggressive responses towards the strange males. Hammour *et al.* [3] found that males which had been separated by a solid stainless steel wall were more aggressive towards a standard opponent than males which have been separated by a perforated zinc wall, and that males separated by a perforated zinc wall are more aggressive than males reared with cage-mates. Kimelman and Lubow [11] reported that exposure to a strange male odor prior to a test reduced aggressiveness. They presented the odor of the same individual for one hour a day from 50 to 100 days of age. Although both authors housed mice individually for more than 50 days, their results suggest that male odor reduces aggressiveness in males.

Olfactory stimuli have another effect on male mice. Some workers have reported that a strange male odor enhances the aggressiveness of adult males [13-14]. Male mice in the present experiment were also isolated in cages stained by strange males from 105 to 119 days of age in order to increase their aggressiveness [15]. Considering the comparison between No-neighbor and Neighbor

males, the male odor may affect conspecifics in different ways according to the age of the recipients and/or duration of exposure to the odor. It is possible that I-mate and Neighbor males become used to a male odor as they grow up, and that a strange male odor may stimulate C-mate and No-neighbor males more effectively than I-mate and Neighbor males, and that this difference produces the dominant-subordinate relationships.

The result of the comparison of C-I and I-C males seems to imply the existence of a sensitive period. A cage-mate before 70 days of age has a greater effect on future aggressiveness than a cage-mate after that age. Similar results were reported by Cairns *et al.* [5]. They found that isolation after 84 days of age is less effective in inducing aggressiveness than isolation from 28, 35 or 56 days of age. Intact male cage-mates before weaning also have a strong effect [9, 16].

Although in the present experiment males reared with a castrated male behaved like individually housed males, their physiological state and the biological implications of rearing with a castrated male are still not fully understood. These issues are currently being investigated.

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